WHAT IS CLAIMED IS:

1. A method for analyzing expression frequencies of genes, which comprises the following steps:

(a) a step of forming a vector primer to which each cDNA is ligated, by annealing the vector primer with each mRNA derived from a cell of which expression frequencies of genes is to be analyzed, and synthesizing the cDNA, said vector primer comprising a linear plasmid vector having a single-stranded poly(T) sequence at one 3′ end, a recognition sequence for a first restriction enzyme in an inner position from the poly(T) sequence, a recognition sequence for a second restriction enzyme near the other end, and a recognition sequence for a type IIS restriction enzyme in an inner position from the recognition sequence for the second restriction enzyme,

(b) a step of digesting the vector primer to which the cDNA is ligated, with the second restriction enzyme and a third restriction enzyme that does not digest the vector primer and forms a digested end of the same shape as a digested end obtained with the second restriction enzyme, to excise an upstream region of the cDNA, and cyclizing the vector primer,

(c) a step of digesting the cyclized vector primer with the first restriction enzyme and the

type IIS restriction enzyme to excise a downstream region of the cDNA so that a tag consisting of a part of the cDNA is left, and cyclizing the vector primer again,
(d) a step of performing polymerase chain reaction (PCR) by using the vector primer as a template and oligonucleotides having nucleotide sequences corresponding to respective flanking regions of the both sides of the tag contained in the vector primer as primers to amplify the tag,
(e) a step of ligating the amplification products to form a concatemer of the tags, and
(f) a step of determining the nucleotide sequence of the concatemer and investigating types and frequencies of tags occurring in the nucleotide sequence.

2. The method according to Claim 1, wherein the ligation reaction in the step (e) is performed in the presence of an adaptor having one end of the same shape as an end of the tag to arrange the adaptor at each end of the concatemer, and the concatemer is amplified by performing PCR using an oligonucleotide having a sequence corresponding to the sequence of the adaptor as a primer.

3. The method according to Claim 1 or 2, wherein, after the step (e), the concatemer is

cloned in a cloning vector for nucleotide sequencing, and then the nucleotide sequence of the concatemer is determined.

4. The method according to any one of Claims 1-3, wherein the recognition sequence for the third restriction enzyme consists of four nucleotides.

5. The method according to any one of Claims 1-4, wherein the vector primer has a recognition sequence for a fourth restriction enzyme of which digestion point is in the same position as or an inner position from the digestion point of the recognition sequence for the second restriction enzyme, which is not excised from the vector primer by the digestion with the type IIS restriction enzyme;

the primer for the downstream side of the tag among the primers used in the step (d) has a recognition sequence for a fifth restriction enzyme that forms an end of the same shape as the end digested with the fourth restriction enzyme; and

the concatemer is formed after the amplified primers are digested with the fourth restriction enzyme and the fifth restriction enzyme.

6. The method according to Claim 5, wherein

the vector primer has a nucleotide sequence different from the recognition sequence for the fifth restriction enzyme by one nucleotide in an inner position from the recognition sequence for the first restriction enzyme, and the nucleotide sequence different by one nucleotide is converted to the recognition sequence for the fifth restriction enzyme by PCR using the primer for the downstream side of the tag.

7. The method according to Claim 6, wherein the third, fourth and fifth restriction enzymes are identical to one another.

8. The method according to any one of Claims 1-7, wherein the vector primer is formed by ligating a linear plasmid obtained by digesting a plasmid having a multicloning site at two sites in the multicloning site, and a partially double-stranded DNA having an end of the same shape as one end of the linear plasmid and a single-stranded poly(T) sequence.